

Fig. 1.

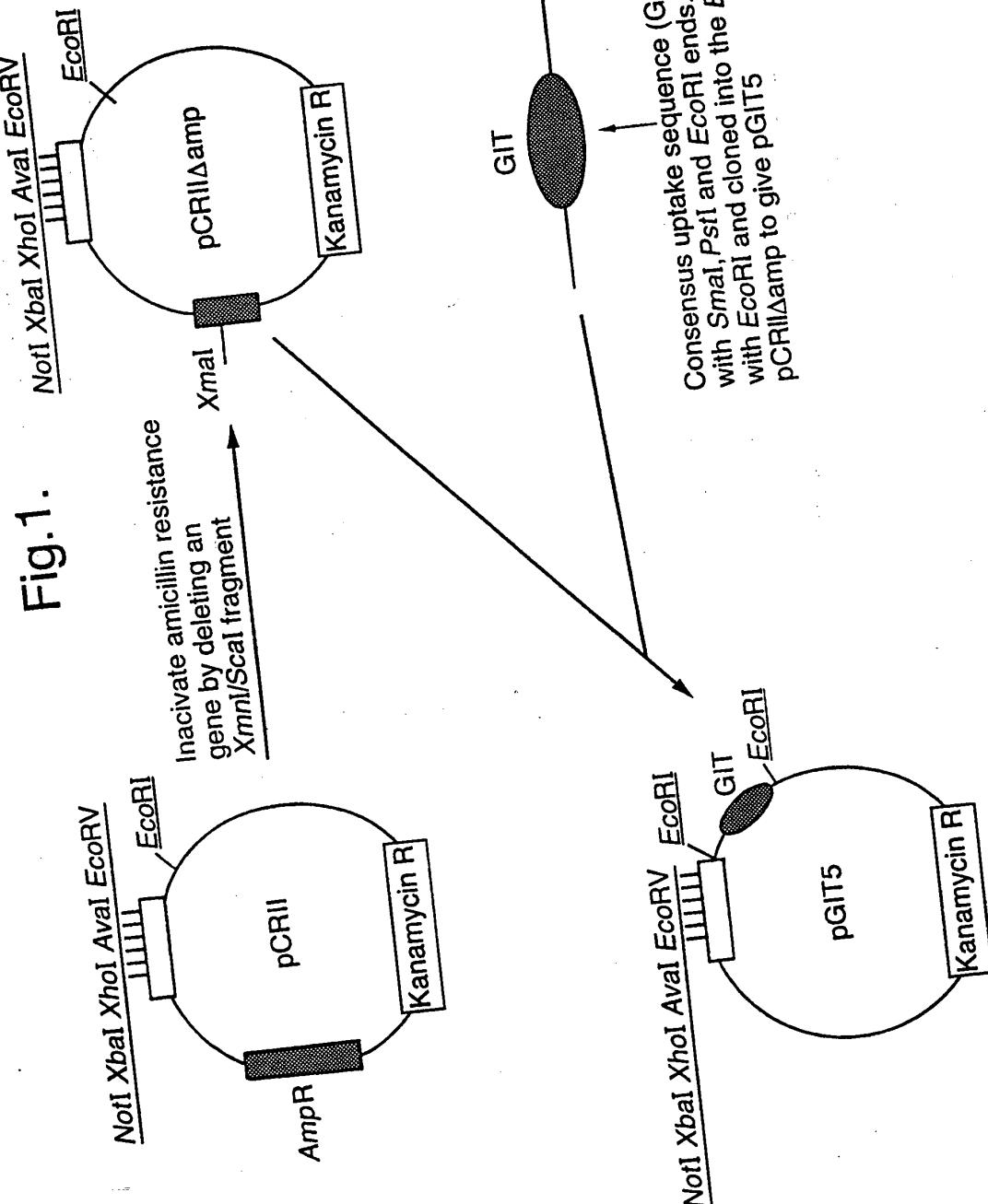
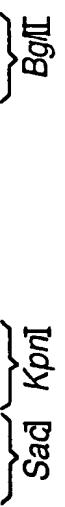


Fig.2.

aroB1-	gcagatccccgaaggctttttataggg 	melting temperature=74°C
aroB2-	gagctcggttaccgtgcagcggttcaggatctgcaag 	melting temperature=72°C
aroB3-	cataaaggatccgttgttgcgcagg 	melting temperature=70°C
aroB4-	ggtaccggctccaaatgaaggcaggatctcgccccc 	melting temperature=74°C

Amplify the two halves of the aroB region by PCR using the above primers in the following combinations

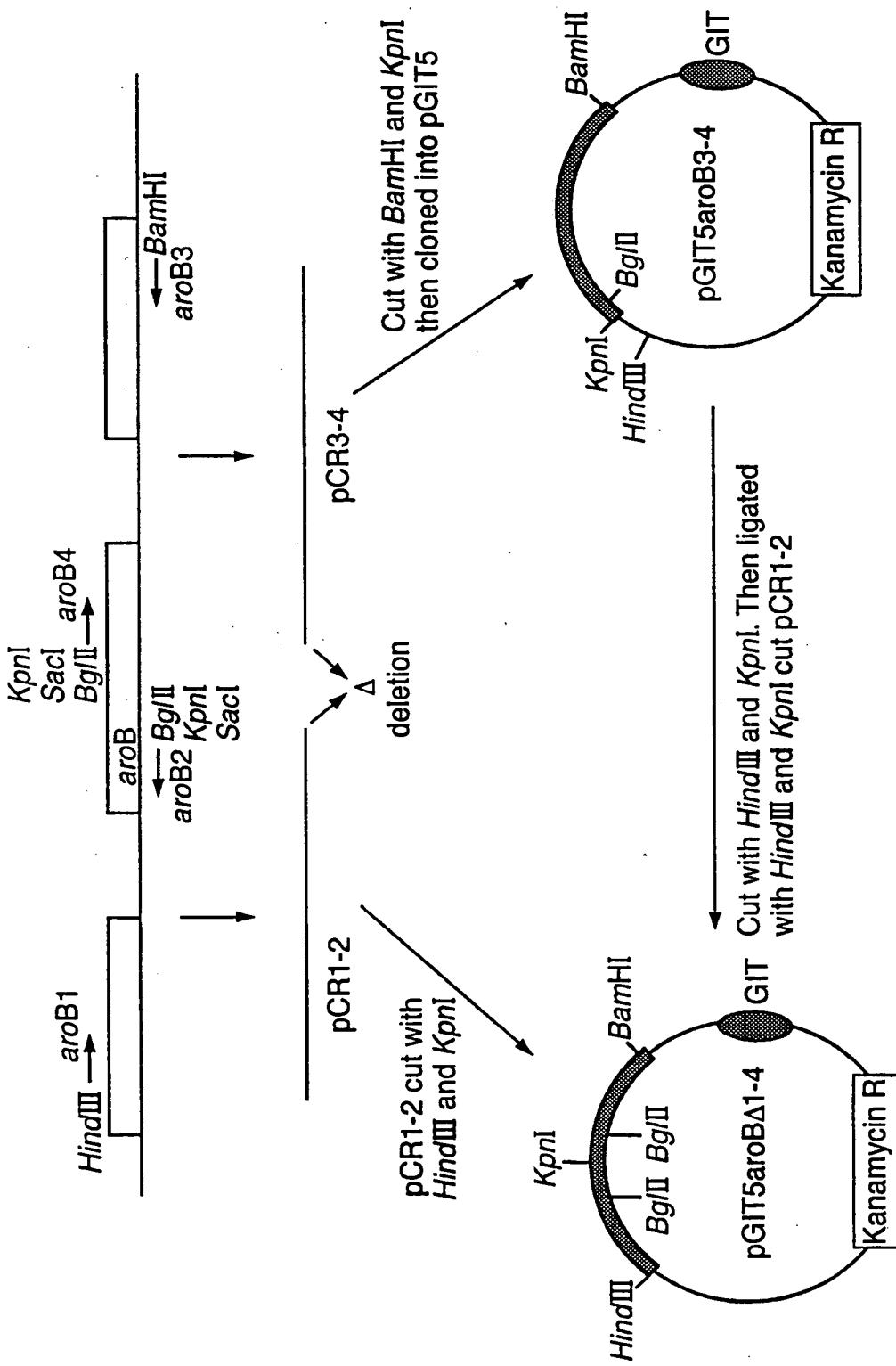
aroB1 + aroB2 - gives a fragment of 1575 bp. From upstream of aroB.

aroB4 + aroB3 - gives a fragment of 1433 bp. From downstream of aroB.

Ligation of these fragments together gives a deletion in the middle of aroB of approximately 150 bp.

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Fig. 3(i).



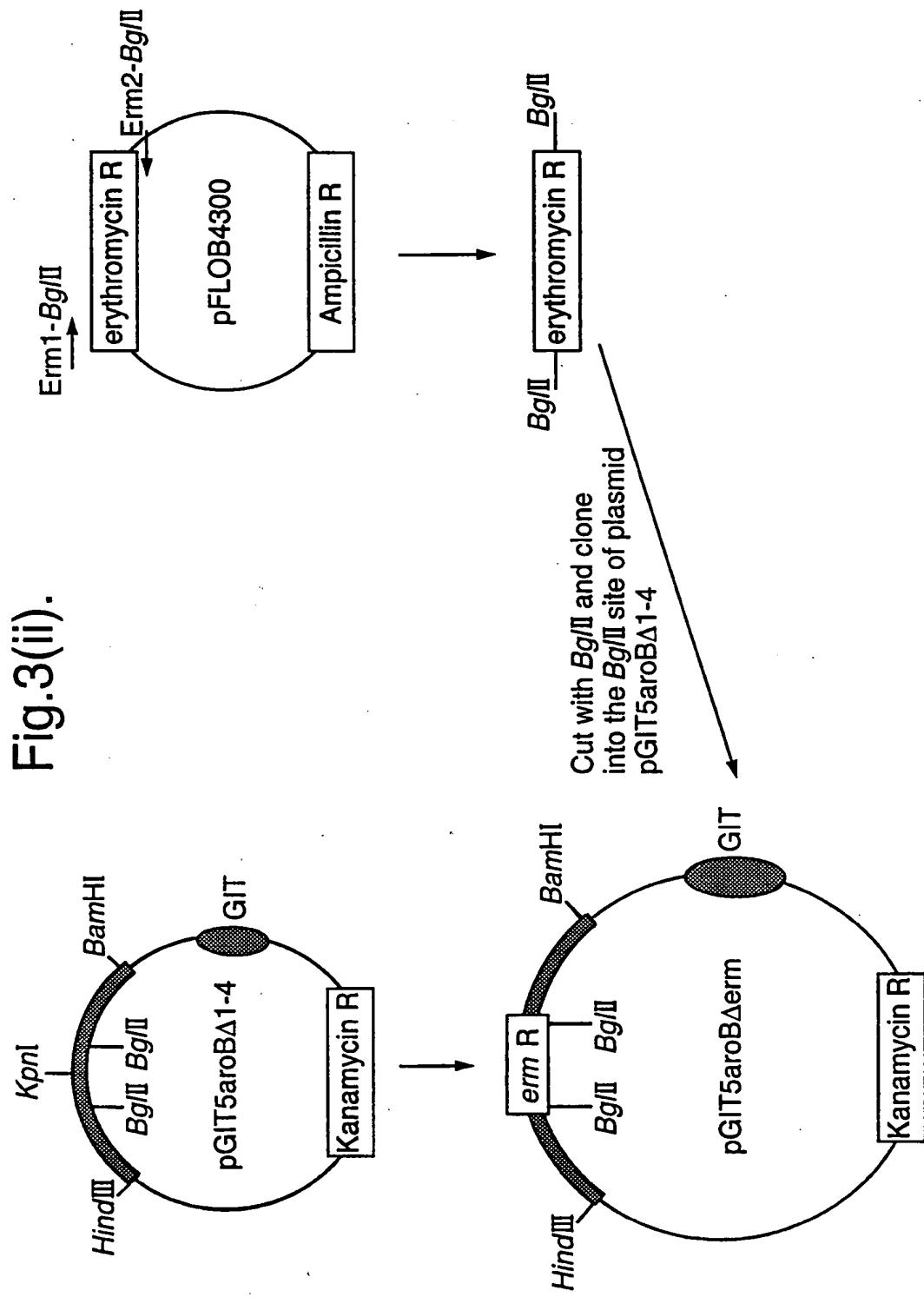


Fig.3(iii).

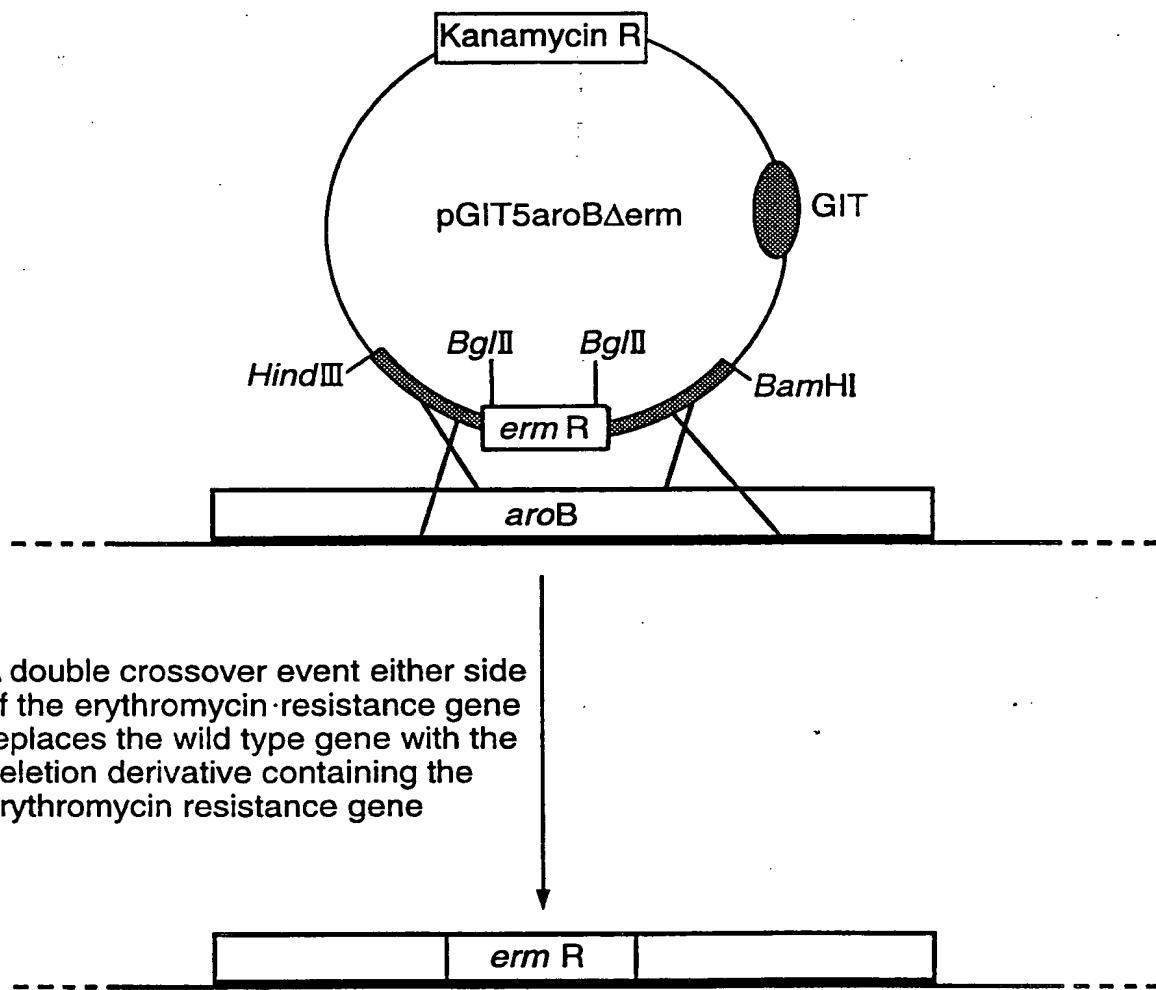
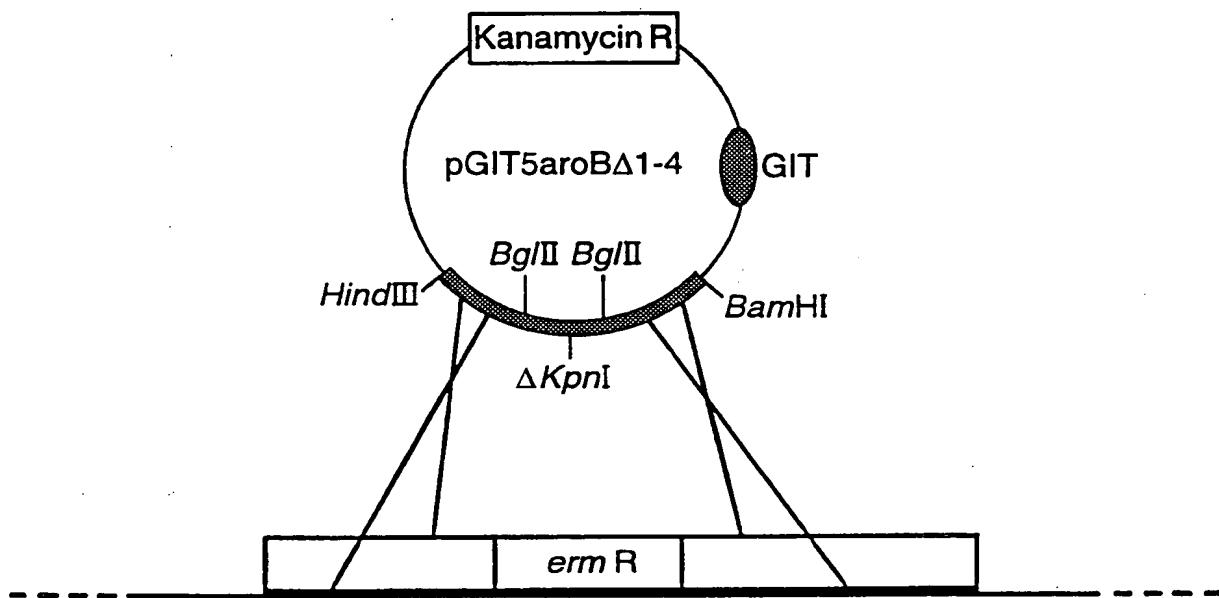
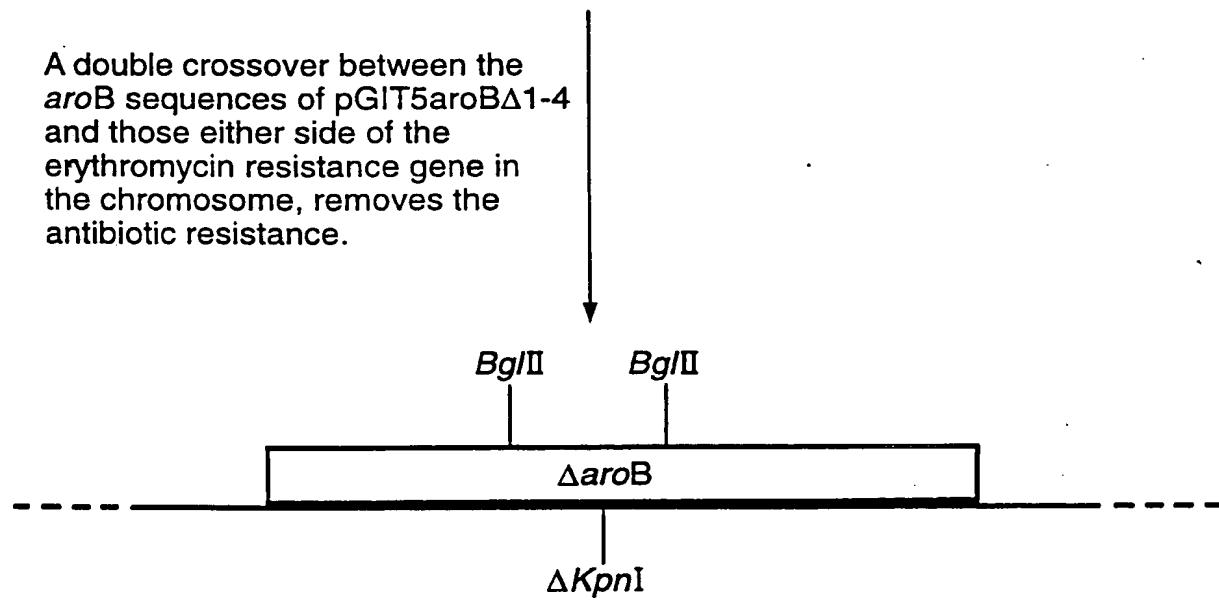


Fig.3(iv).



A double crossover between the *aroB* sequences of pGIT5aroBΔ1-4 and those either side of the erythromycin resistance gene in the chromosome, removes the antibiotic resistance.



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Fig.4.

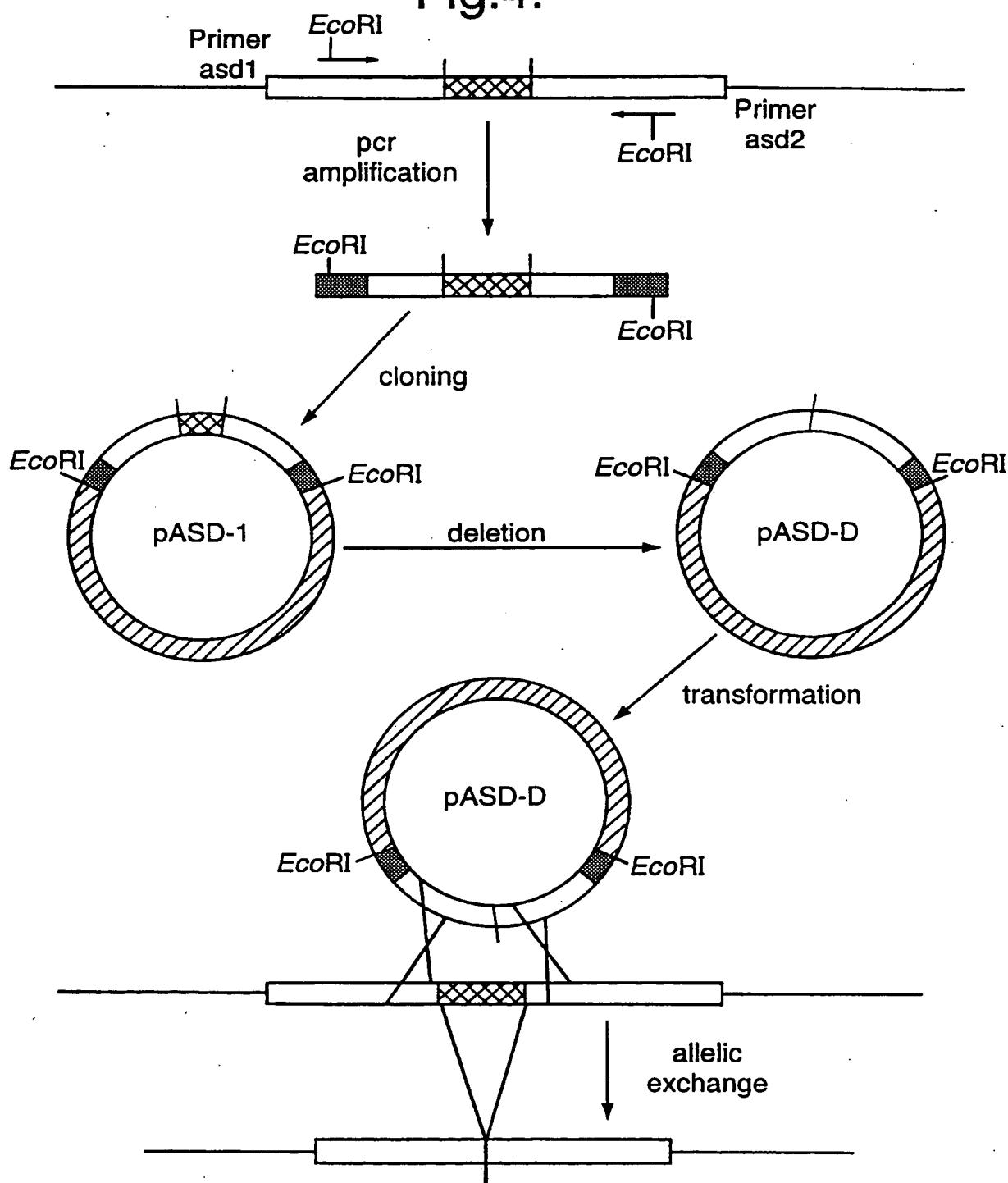
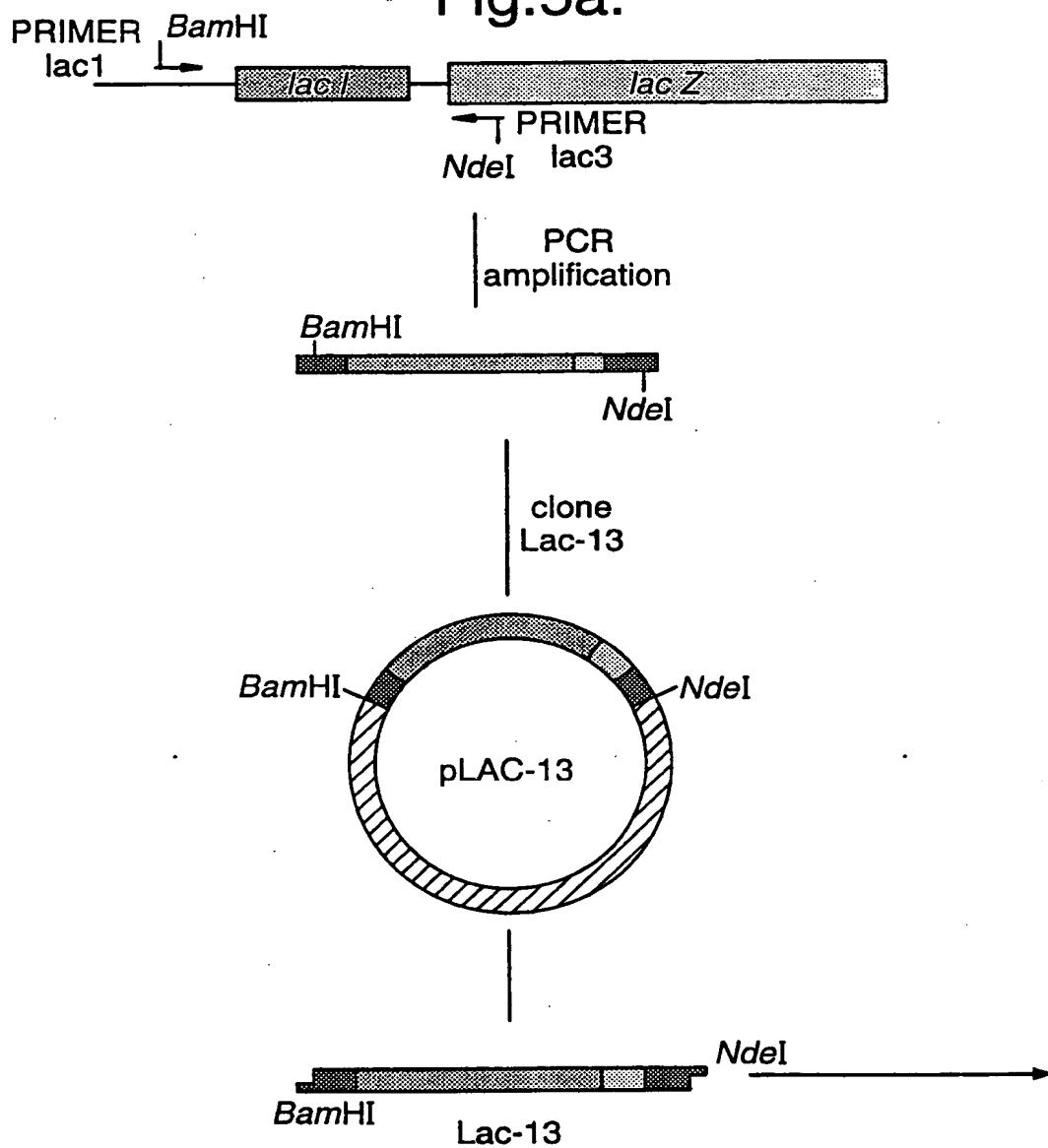
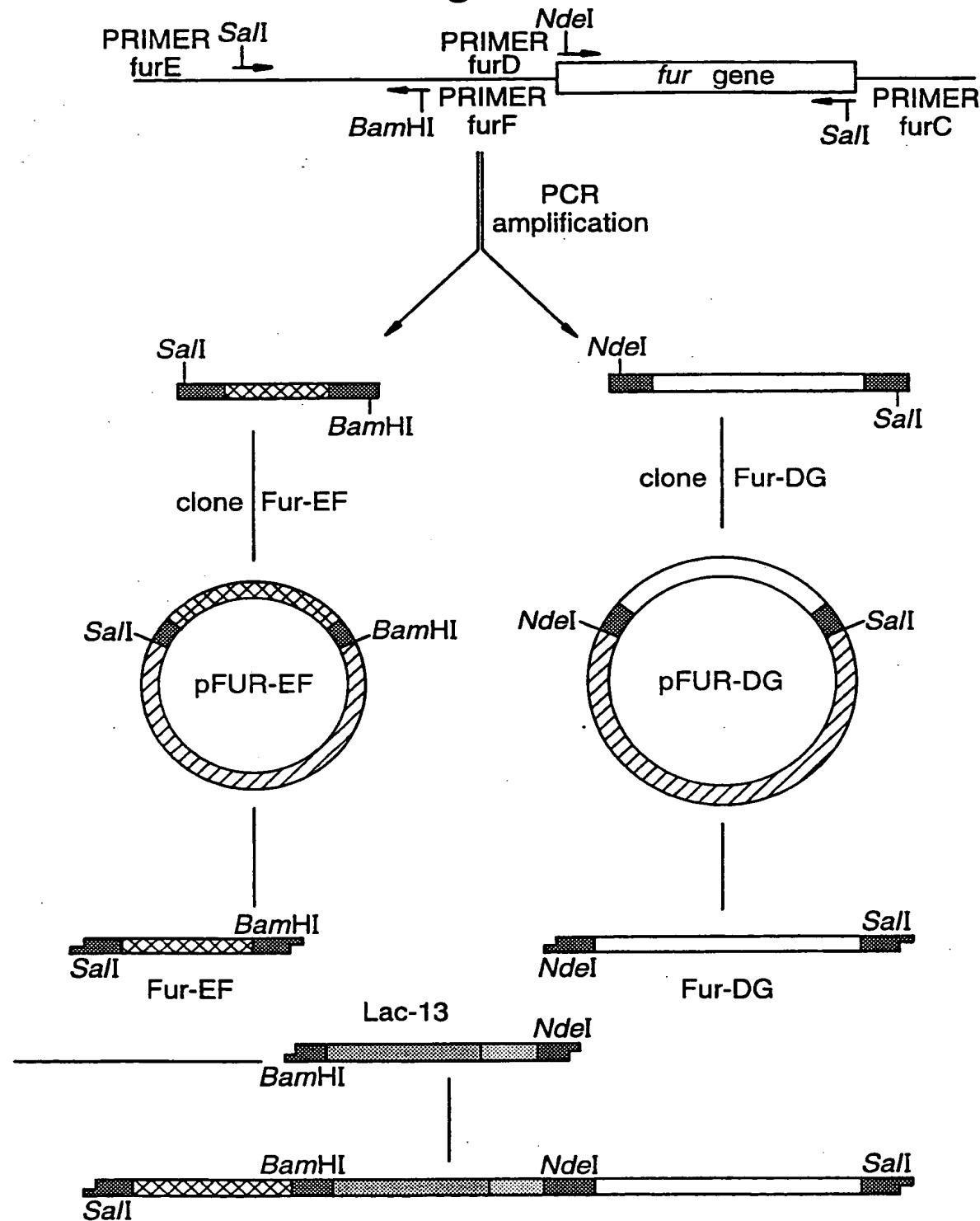


Fig.5a.



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Fig.5b.



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